

Specific immunity to human papilloma virus (HPV) in patients with genital warts

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SUMMARY A study of human papilloma virus (HPV) specific cellular and humoral immunity in 30 patients with genital warts is reported. By *in vivo* testing with purified, inactivated plantar wart virus, a cell-mediated immunity to HPV was determined in 60% of patients. Circulating antibodies, evaluated by immunofluorescence testing, were rare, but these increased after an intradermal test had been carried out, especially in patients with a positive skin test, suggesting a booster effect. No significant difference was found between this group of patients and those having skin warts. Our results showed a specific immune response to HPV in most patients, confirming the role of the viral agent in the induction of genital warts.

Introduction

The viral aetiology of genital warts has now been well established by the presence of virus particles with typical human wart virus morphology (Dunn and Ogilvie, 1968; Oriol and Almeida, 1970) in these lesions. However, there is no evidence as yet of antigenic identity between the viruses found in different clinical types of wart in man (Almeida *et al.*, 1969) and very few studies have been undertaken on specific immunity to human papilloma virus (HPV) in patients with genital warts. Most studies carried out on humoral immunity have been on patients suffering from skin warts (Ogilvie, 1970; Cubie, 1972; Pyrhönen and Penttinen, 1972) and specific cellular immunity studies have been performed using only *in vitro* tests (Morison, 1974; Ivanyi and Morison, 1976; Lee and Eiseinger, 1976). This study was therefore aimed at determining the levels of specific humoral and cellular immunity in patients with genital warts before and after an *in vivo* test (intradermal test), using purified, inactivated HPV prepared from plantar warts, and comparing them with results obtained in a normal infected population (Thivolet *et al.*, 1977).

Material and methods

PATIENTS

The group studied comprised 25 Caucasians and 5 Arabs (19 males, 11 females). They had had genital warts for an average period of five months (minimum 1 month, maximum 15 months). None had a history of skin warts. Their average age was 26 years (minimum 16 years, maximum 55 years).

Forty adults and 23 children with various dermatoses but no clinical evidence or history of cutaneous viral warts were used as controls.

VIRUS PURIFICATION

HPV was obtained from plantar warts, acquired after surgical excision, immediately frozen in liquid nitrogen and stored at -25°C . This HPV was purified according to the procedure described by Pass and Marcus (1973) and inactivated with 0.4% formalin, dialysed against phosphate buffer solution (PBS), and diluted to approximately 10^{10} particles per 0.1 ml (5 μg proteins). Controls were set up according to a method previously described (Thivolet *et al.*, 1977) (Fig. 1).

INTRADERMAL TEST (IDT)

The patients were injected intradermally with 0.1 ml HPV suspension containing 5 μg virus protein. They were examined after 24, 48, and 72 hours. Erythema

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and induration were considered as indications of a positive test after a minimum of 24 hours.

HUMORAL IMMUNITY TEST

Sera were taken from patients just before IDT and eight days after. Indirect immunofluorescence (IF) tests were then performed as described by Genner (1971) (Fig. 2). In these tests sheep antihuman IgG and antihuman IgM conjugates (Behring-Hoechst) were used after absorption and dilution. Frozen sections of plantar warts, which showed specific nuclear fluorescence with a rabbit HPV antiserum (Viac *et al.*, 1977b) were used to detect antibodies in the patients' sera. The specificity of the IF test was shown by the absence of staining in a pre-immune rabbit serum and in human sera derived from children under the age of five years, who did not have warts. Sections of normal adult skin, exposed to the positive sera, also failed to yield fluorescence. All sera were tested at a dilution of 1:10 and slides were viewed with a Leitz fluorescence microscope (epi-illumination Orthoplan).

STATISTICAL ANALYSIS

Comparison between the observed percentages was based on the χ^2 test and the degree of significance was derived from the χ^2 table of Fisher and Yates.

Results

CELLULAR IMMUNITY TEST

The percentage of positive IDT in patients with genital warts is shown in Table 1. This percentage (60%), similar to that obtained from patients with plantar warts (64.3%), is higher than that seen in patients with hand (42.9%) or flat warts (41.8%). However, there is no statistical difference between these different values. The χ^2 values between genital warts and hand or flat warts were respectively 2.29 and 1.74. The percentage of positive IDT among all patients with different types of warts (52.5%) is

Table 1 IDT in patients with genital warts: comparison with patients with other types of warts and with controls

Type of case	No. of cases	IDT positive	Positive (%)
Warts			
Genital	30	18	60.0
Plantar	30	19	64.3
Hand	49	21	42.9
Flat (face or hand)	12	5	41.8
Controls			
Children aged 1-5 years	23	0	0.0
Others	40	4	10.0
Total	63	4	6.3

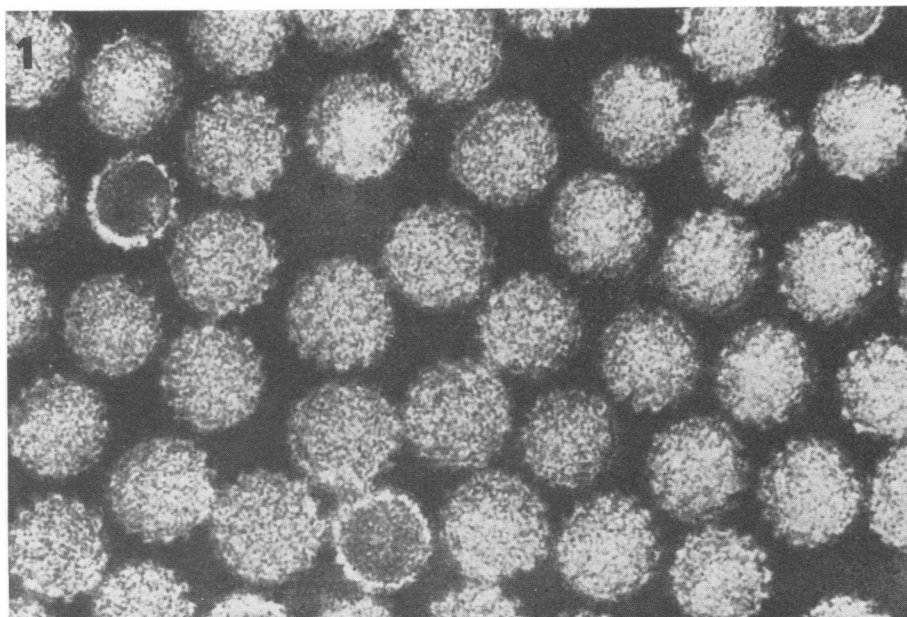


Fig. 1 Electron micrograph of HPV particles purified according to the procedure described by Pass and Marcus (1973) and observed after negative staining with 2% phosphotungstic acid buffered to pH 7.0 $\times 250\ 000$

statistically higher than that of the control group (6.3%) (χ^2 value = 36.2). The control group comprised 40 adults, of whom four showed positive IDT, and 23 children aged 1-5, all of whom had negative results.

HUMORAL IMMUNITY TEST

The incidence of circulating antibodies was low before IDT (27.6%) but increased significantly after the skin test, to a value of 75% (χ^2 value = 11; $P=0.0001$). Most of the antibodies detected were of the IgM class (Table 2). Patients with a positive result to the skin test had a better seroconversion (86.6%) than patients with a negative result (55.5%); they also had a spontaneous incidence of antibodies greater than the negative group (Table 3).

Table 2 Incidence of circulating antibodies (IF test) in patients with genital warts

Time	No. of sera	Positive sera			P level
		IgG	Total (IgM, IgG)	Positive (%)	
Before IDT	29	2	8	27.6	0.001
After IDT	24	7	18	75.0	

Discussion

A few studies have been carried out on specific immunity in patients with genital warts. Cell

Table 3 Correlation between IDT and circulating antibodies

IDT	Before skin test			After skin test		
	No. of sera		Positive (%)	No. of sera		Positive (%)
	Positive	Total		Positive	Total	
Positive	7	19	36.9	13	15	86.6
Negative	1	10	10.0	5	9	55.5

mediated immunity to HPV has been studied by *in vitro* tests (Morison, 1974; Ivanyi and Morison, 1976), but there is much controversy regarding the significance of these tests (Lee and Eiseinger, 1976) and, in general, patients with anogenital warts were excluded from these studies (Morison, 1974).

Other authors (Maderna, 1934; Biberstein, 1944) tested skin hypersensitivity in patients with genital warts, but they used a saline extract of wart tissue, rather than purified HPV.

In this study, cellular immunity to wart virus has been tested directly by an IDT, using purified inactivated HPV. As already shown, the skin test we used seemed to be specific and sensitive in preliminary sera (Thivolet *et al.*, 1977). The incidence of positive IDT in patients with genital warts was as high as in patients with plantar warts and showed the existence of a cell-mediated immunity to HPV within these patients.

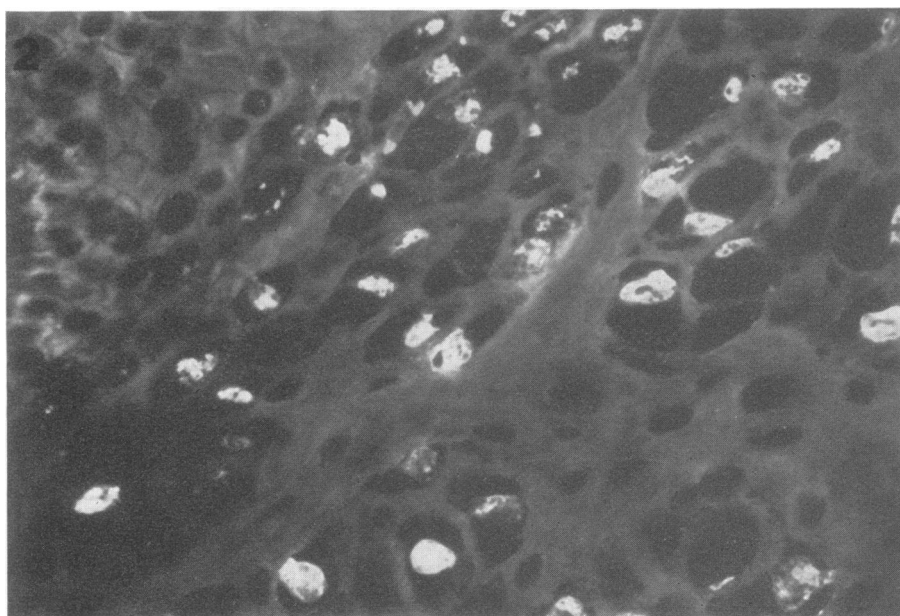


Fig. 2 Cryostat section of plantar wart. $\times 40$. Nuclei containing viral antigen. Stained with sheep antihuman IgG conjugate

Serological studies have demonstrated the existence of wart virus specific antibodies in the sera of patients with genital warts (Ogilvie, 1970). However, Almeida *et al.* (1969) found a one-way cross-reaction in such sera, which reacted with virus only from genital warts and not with that from skin warts, while sera from skin warts reacted with both types of virus, but more strongly with the homologous virus. In our study, a few sera (27.6%) had antibodies detectable by IF testing on plantar warts; this small percentage can be explained by the difference in antigenic affinity between plantar and genital wart viruses, or by the lower antigenic stimulus received in such patients, due to the lower number of virus particles present in genital warts (Ogilvie, 1970). The seroconversion observed after the skin test (27.6–75%), especially in patients with a positive skin test (36.9–86.6%) was in favour of wart virus specific antibodies present at low levels in many cases.

Thus, genital warts induced a specific immune response to HPV: a cell mediated immunity which seemed to play a major role in the cure of skin warts (Viac *et al.*, 1977a) and, less frequently, wart virus specific antibodies, which increased significantly after the skin test, thus acting as a booster effect.

Our results were similar to those obtained with patients bearing skin warts (Viac *et al.*, 1977b) and confirmed the existence of a host immune response directed toward the genital wart inducing viral agent (HPV).

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